RESEARCH

Habitual physical activity is associated with lower fasting and greater glucose-induced GLP-1 response in men

Charlotte Janus[1,2], Dorte Vistisen[3], Hanan Amadid[3,4], Daniel R Witte[2,4,5], Torsten Lauritzen[4], Søren Brage[6], Anne-Louise Bjerregaard[4], Torben Hansen[7], Jens J Holst[1,7], Marit E Jørgensen[3,8], Oluf Pedersen[7], Kristine Færch[3] and Signe S Torekov[1,7]

[1]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[2]Danish Diabetes Academy, Odense University Hospital, Odense, Denmark
[3]Steno Diabetes Center Copenhagen, Gentofte, Denmark
[4]Department of Public Health, Research Unit of Epidemiology, Aarhus University, Aarhus, Denmark
[5]Steno Diabetes Center Aarhus, Aarhus, Denmark
[6]MRC Epidemiology Unit, University of Cambridge, Cambridge, UK
[7]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark
[8]National Institute of Public Health, University of Southern Denmark, Odense, Denmark

Correspondence should be addressed to S S Torekov: torekov@sund.ku.dk

Abstract

Rationale: The hormone glucagon-like peptide-1 (GLP-1) decreases blood glucose and appetite. Greater physical activity (PA) is associated with lower incidence of type 2 diabetes. While acute exercise may increase glucose-induced response of GLP-1, it is unknown how habitual PA affects GLP-1 secretion. We hypothesised that habitual PA associates with greater glucose-induced GLP-1 responses in overweight individuals.
Methods: Cross-sectional analysis of habitual PA levels and GLP-1 concentrations in 1326 individuals (mean (S.D.) age 66 (7) years, BMI 27.1 (4.5) kg/m^2) from the ADDITION-PRO cohort. Fasting and oral glucose-stimulated GLP-1 responses were measured using validated radioimmunoassay. PA was measured using 7-day combined accelerometry and heart rate monitoring. From this, energy expenditure (PAEE; kJ/kg/day) and fractions of time spent in activity intensities (h/day) were calculated. Cardiorespiratory fitness (CRF; mL O_2/kg/min) was calculated using step tests. Age-, BMI- and insulin sensitivity-adjusted associations between PA and GLP-1, stratified by sex, were evaluated by linear regression analysis.
Results: In 703 men, fasting GLP-1 concentrations were 20% lower (95% CI: −33; −3%, $P = 0.02$) for every hour of moderate-intensity PA performed. Higher CRF and PAEE were associated with 1–2% lower fasting GLP-1 ($P = 0.01$). For every hour of moderate-intensity PA, the glucose-stimulated GLP-1 response was 16% greater at peak 30 min (1; 33%, $P_{rAUC0\text{-}30} = 0.04$) and 20% greater at full response (3; 40%, $P_{rAUC0\text{-}120} = 0.02$). No associations were found in women who performed PA 22 min/day vs 32 min/day for men.
Conclusion: Moderate-intensity PA is associated with lower fasting and greater glucose-induced GLP-1 responses in overweight men, possibly contributing to improved glucose and appetite regulation with increased habitual PA.

Key Words

- habitual physical activity
- glucagon-like peptide-1 (GLP-1)
- overweight
- prediabetes
- exercise

Endocrine Connections (2019) **8**, 1607–1617

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-19-0408

Introduction

Obesity and type 2 diabetes (T2D) are among the leading causes of death and overall health complications such as hypertension, dyslipidaemia, and heart disease (1). However, another strong predictor of such complications is a sedentary lifestyle, which constitutes an independent health risk (2). In high-income countries, the second most important preventable cause of premature death is physical inactivity, next to smoking (2, 3). A recent meta-analysis of data from studies examining the relationship between accelerometer-measured physical activity and all-cause mortality demonstrated that higher levels of total physical activity, at any intensity, and less time spent sedentary, are associated with substantially reduced risk for premature mortality (4).

Glucagon-like peptide-1 (GLP-1) is a peptide hormone secreted from intestinal L-cells upon meal intake (5). GLP-1 stimulates insulin secretion in a glucose-dependent manner – as part of 'the incretin effect' – being responsible for up to 70% of the postprandial insulin response in healthy individuals whilst being severely impaired in patients with prediabetes and T2D (5, 6). Furthermore, GLP-1 responses are lower in individuals with overweight and obesity, independently of glucose tolerance status (6). Interestingly, the blunted GLP-1 response in obesity normalizes after sustained weight loss (7). Besides its glycaemic effects, GLP-1 inhibits appetite, reduces food intake, and slows gastric emptying (8, 9).

Dependent on intensity, physical activity causes mechanical bouncing, changes neuroendocrine activity, and shifts blood flow away from the gastrointestinal tract towards the lungs and working muscles (10, 11). These changes may affect gastrointestinal and digestive functions such as motility, absorption, and secretion. During exhausting endurance exercise (e.g. long-distance running), this can lead to unpleasant symptoms such as diarrhea and intestinal cramps (10). At low-to-moderate intensity, however, physical activity seems to have beneficial effects on gastrointestinal health by reducing risks of constipation (12, 13), which is strongly related to inactivity (14). Also the effects of exercise on secretion of gastrointestinal hormones like GLP-1 have been investigated (15). In normal-weight adults, postprandial GLP-1 responses are suggested to increase after acute exercise (16, 17, 18, 19, 20) whereas studies in individuals with overweight show contradicting results of unaffected, increased, or reduced GLP-1 responses after acute exercise (17, 21, 22, 23, 24, 25, 26, 27). The contradicting findings between studies are likely due to different participant characteristics (e.g. BMI, age, and fitness level), exercise protocols (e.g. duration and intensity), and GLP-1 measurement techniques (28). For instance, free fatty acids are found to inhibit the secretion of GLP-1 (29, 30) and therefore differences in plasma free fatty acid responses to acute exercise might explain some differences in the effects of exercise on GLP-1 secretion.

It may be asked whether one or few bouts of structured exercise are relevant in the overall regulation of circulating biomarkers like GLP-1. Moreover, since the risk of developing T2D is strongly associated with overweight and excess body fat (31), focusing on daily physical activities that are less vigorous and weight bearing (e.g. to the knee joints) compared to structured exercise sessions might be a more achievable strategy in relation to T2D prevention. Therefore, our objective was to investigate whether an association between habitual physical activity and GLP-1 secretion can be demonstrated in an overweight population at risk of developing T2D. Since greater leisure time physical activity is associated with substantially lower incidence of T2D (32) and because greater GLP-1 responses to glucose are associated with better beta-cell function through stimulation of insulin secretion (6), we hypothesised that more time spent being physically active is positively associated with glucose-induced GLP-1 responses from the intestinal L-cells, independently of insulin sensitivity. To test our hypothesis, we investigated the associations of fasting and glucose-stimulated GLP-1 concentrations with moderate-intensity physical activity, physical activity energy expenditure (PAEE) as a measure of total activity volume, and cardiorespiratory fitness level (CRF) in an elderly population of 1326 individuals with BMI ranging from normal-weight to obese. This is, to our knowledge, the first investigation of the association of habitual physical activity with GLP-1 responses in a large population at risk of developing T2D.

Methods

Ethics

The study was conducted according to the Helsinki Declaration and approved by the Ethics Committee of the Central Denmark Region (ref. no. 20080229). All participants provided oral and written informed consent before participation in the study.

Study population

This present study is an analysis of data from the Danish ADDITION-PRO study described in detail elsewhere (33).

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https://doi.org/10.1530/EC-19-0408

The study includes a subpopulation of participants from previously published studies (6, 34, 35, 36). The ADDITION-PRO study is a longitudinal cohort follow-up study of the Danish part of the ADDITION-Europe study in which a total of 2082 individuals with impaired glucose regulation at screening and individuals from a random subsample of individuals at lower diabetes risk completed a health examination from 2009 to 2011.

In the present analysis, we excluded participants with known diabetes ($n=336$), those who were fasting less than 8 h prior to the health examination ($n=20$), those who could not be classified due to missing information on fasting or 2-h plasma glucose concentrations ($n=12$), those with no blood samples taken for measurement of GLP-1 ($n=252$), and those with no measurements of physical activity ($n=136$), leaving a subpopulation of 1326 individuals. Of the 1326 included individuals, 793 (60%) had their cardiorespiratory fitness measured.

General information and body composition

The Danish civil registration number provided information on age and sex, whereas information on smoking status (current smoker, never smoker, ex-smoker) and alcohol consumption (units per week) was obtained from general health questionnaires completed during the health examination (33). Height was measured without shoes to the nearest 0.1 cm on a stadiometer (Seca, Hamburg, Germany). Participants were weighted to nearest 0.1 kg with light clothes and without shoes using a body composition analyser (Tanita, Tokyo, Japan). Waist circumference was measured at the mid-point between the lower costal margin and the level of the anterior superior iliac crest. The measurement was taken twice by the same person to nearest 0.1 cm, and the mean value of the two measurements was used.

Physical activity assessment

An objective measure of physical activity behaviour was obtained from individually calibrated heart rate and uniaxial accelerometers (ActiHeart, CamNTech, Cambridge, UK) (37), which the participants wore for 7 consecutive days. Heart rate and accelerometry data were downloaded using the manufacturer's software (www.camntech.com). Heart rate data were pre-processed to eliminate noise (38) and calibrated to physical activity energy expenditure (PAEE) using a submaximal step test ($n=793$) as described in detail elsewhere (39) or a group equation for those without step test ($n=533$) (34).

Physical activity intensity was modelled using a branched equation framework (37) from which total physical activity energy expenditure (PAEE) and fractions of time spent at different physical activity intensity levels were derived. A full description of the processing of accelerometer data and heart rate measures from the combined monitor is available elsewhere (38). Intensity was expressed as multiples of metabolic equivalent of tasks (METs) using a standard value for resting metabolic rate (71 J/min/kg) and defined as the following intensity categories: sedentary behaviour (<1.5 METs), light intensity physical activity (LPA) (1.5-3.0 METs), and moderate-to-vigorous intensity (MVPA) (>3.0 METs).

Heart rate data were preprocessed using a two-stage Gaussian Process Robust regression to de-noise the heart rate signal according to the method described by Stegle *et al.* (38). The procedure works well for dealing with noise when the sensor is worn and, owing to the short-term covariance function, also for brief periods of missing data (e.g., electrode changes) (35). Non-wear time was identified using the Bayesian uncertainty estimate from a Gaussian Process Robust regression as described by Stegle *et al.* (38) and defined as prolonged periods of inactivity combined with non-physiological heart rate. Such segments were marked as non-wear if longer than 90 min as described by Amidid *et al.* (36). All measures were summarized to daily averages whilst minimising diurnal bias caused by imbalance in non-wear patterns; this technique provides estimates of PAEE comparable to gold-standard isotopic assessment (40).

Cardiorespiratory fitness measurement

A submaximal 8-min step test was performed in order to estimate cardiorespiratory fitness (CRF) (expressed as ml O_2/kg/min) by extrapolating the linear regression line between the observed heart rate and oxygen cost of each step (39) to maximal heart rate defined by the Tanaka equation (208-0.7 × age). Participants had to complete a minimum of 4 min of the step test to be included in analysis.

The following physical activity parameters were included in the present study: physical activity energy expenditure (PAEE; kJ/kg/day), moderate-to-vigorous intensity physical activity (MVPA; hours/day), and cardiorespiratory fitness (CRF; mL O_2/kg/min).

Oral glucose tolerance test

Participants met in the morning after an overnight fast of minimum 8 h and venous blood samples were drawn.

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Subsequently, the participants underwent a standardized oral glucose tolerance test (OGTT), ingesting 75 g glucose dissolved in 250 mL water, and blood samples were drawn 30 and 120 min after glucose intake.

Biochemical measures

Following preanalytical guidelines for measurement of GLP-1 (41), plasma samples were taken before and during the 2-h OGTT in chilled EDTA-coated tubes, put on ice immediately, and centrifuged within 30 min for 10 min (3000 rpm at 4°C). Subsequently, plasma was isolated and stored at −80°C. Concentrations of GLP-1 were analysed using a validated in-house developed RIA. The assay is COOH-terminal and thus measures both the active form of GLP-1 (7-36)NH2 and the DPP-4 generated metabolite GLP-1 (9-36)NH2 to quantify total GLP-1. The analytical detection limit was 1 pmol/L, and intra- and interassay coefficients of variation were 6 and 1–5%, respectively, at GLP-1 plasma concentrations of 20 pmol/L. The samples were analysed consecutively within 2 months using identical quality controls and identical batches for all reagents in each analysis set.

Insulin concentrations in serum (prepared by keeping whole blood at room temperature for 0.5–1.5 h followed by centrifugation for 10 min at 3000 rpm without cooling) was measured by immunoassay. Plasma glucose (prepared immediately upon collection in fluoride-heparin coated tubes, placed on ice, and centrifuged for 10 min at 3000 rpm at 4°C) was measured by HPLC as described fully elsewhere (33).

Calculations

GLP-1 responses were calculated as total areas under the curve (tAUCs, pmol/L × min) from the fasting state (baseline) to 30 and 120 min by the use of the trapezoid rule. From these, we calculated the relative peak response ($rAUC_{0–30}$) as $tAUC_{0–30}$/(fasting concentration × 30 min) and the relative full response ($rAUC_{0–120}$) as $tAUC_{0–120}$/(fasting concentration × 120 min). Because the response of GLP-1 to oral glucose peaks around 30 min (42) the estimated $rAUC_{0–30}$ (GLP-1 peak 30 min) is likely to include the peak GLP-1 response, whereas the $rAUC_{0–120}$ will reflect the full GLP-1 response to the 2-h OGTT. The rAUC reflects the change in GLP-1 concentrations relative to baseline (fasting) level, that is, rAUC > 1 indicates an increase in GLP-1 levels from fasting levels, whereas rAUC < 1 indicates a decrease in GLP-1 levels. The rAUC is always positive and can therefore be logarithmically transformed, which is not the case for the incremental area under the curve calculated as the difference between tAUC and baseline. The relative and incremental AUCs express the same (the change in GLP-1 release from baseline) but on a different scale (relative vs absolute).

As a proxy measure of peripheral insulin sensitivity, we calculated the insulin sensitivity index ($ISI_{0–120}$) (43). As a surrogate measure of first-phase insulin release, we calculated the insulinogenic index as (insulin_30 min − insulin_0 min)/(glucose_30 min − glucose_0 min) (44).

Statistical analyses

Fasting plasma GLP-1 and relative responses of GLP-1 (peak: $rAUC_{0-30}$ and full: $rAUC_{0-120}$) were considered as outcomes. The following measures of physical activity were considered as exposures: physical activity energy expenditure (PAEE), moderate-to-vigorous intensity physical activity (MVPA), and cardiorespiratory fitness (CRF).

Associations of GLP-1 response and physical activity measures (PAEE, MVPA, and CRF) were studied by linear regression analyses. All analyses in the present study were stratified by sex because a sex-difference in terms of GLP-1 response has previously been found in this study cohort (6). Analyses were adjusted for age (model 1) and further by BMI and ISI_{0-120} (model 2) because previous studies have found a positive association between insulin sensitivity and GLP-1 response (6, 45) and between PAEE and insulin sensitivity (34). In model 2, we tested for a modifying effect of ISI_{0-120} on the associations with physical activity by including interaction terms between ISI_{0-120} and the physical activity exposures in the model. For MVPA, we further adjusted for PAEE to ensure that an increase in MVPA was at the expense of a reduction in LPA or sedentary behaviour and not due to an increase in PAEE.

Data on $ISI_{0–120}$ and GLP-1 responses were logarithmically transformed before analysis to fulfill the requirement of a normal distribution of the model residuals. A two-sided 5% level of significance was used.

In a sensitivity analysis, we repeated the analyses above for the subset of participants with cardiorespiratory fitness data available ($n = 793$).

Statistical analyses were performed in R, version 3.6.0 (The R Foundation for Statistical Computing) and SAS, version 9.4 (SAS Institute).

Descriptive statistics are presented as mean ± SD for normally distributed variables and medians (interquartile

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https://doi.org/10.1530/EC-19-0408

range) for non-normally distributed variables. Outcome data (fasting and glucose-stimulated responses of GLP-1) are presented as percentage change (%) with 95% CI by a unit increase in the physical activity parameters.

Results

Characteristics of the study population

The mean age of the 1326 participants (53% men) was 66±7 years and mean BMI was 27.1±4.5 kg/m^2 (Table 1). 99.2% of the participants had a minimum of 24 h of ActiHeart wear time and 97% had a minimum of 48-h wear time. The 703 men spent 0.54 h/day (0.22; 1.02) at moderate-to-vigorous physical activity (MVPA; >3.0 METs) compared to 0.37 h/day (0.16; 0.76) for the 623 women (Table 1), corresponding to 32 min/day and 22 min/day, respectively. 44% of the men and 39% of the women met the Danish guidelines on moderate-intensity physical activity (i.e. ≥30 min/day) (Table 1) (46). Men also had higher cardiorespiratory fitness levels (CRF) (30.8±5.4 mL O_2/kg/min) than women (28.7±5.1 O_2/kg/min) (Table 1). More than 98% of the included participants spent time in MVPA. Noteworthy, however, almost none of the participants spent time performing vigorous intensity PA (VPA; >6.0 METs) (0.00 min/day (0.00; 0.43)) (Table 1).

Excluded participants did not differ in terms of age and sex (Supplementary Table 1, see section on supplementary data given at the end of this article). However, they were more likely to smoke, were slightly more overweight, and almost half of them had known T2D. There was no modifying effect of peripheral insulin sensitivity ($ISI_{0\text{-}120}$) on the associations between fasting GLP-1 or glucose-stimulated response GLP-1 and PA ($P \geq 0.084$). Therefore, the interaction term was removed from the models.

Fasting levels of GLP-1

In men, but not in women, fasting levels of GLP-1 were 19.5% lower (−33.0; −3.3%, $P=0.021$) for every 60-min

Table 1 Baseline characteristics of the study population.

	n	Total	Women	Men
n		1326	623	703
Age (years)	1326	66 (7)	66 (7)	66 (7)
Current smokers (%)	1326	15.8 (13.9; 17.9)	13.3 (10.8; 16.2)	18.1 (15.3; 21.1)
Glucose tolerance status (%)	1326			
NGT	–	53.0 (50.3; 55.7)	58.9 (54.9; 62.8)	47.8 (44.0; 51.6)
Pre-diabetes	–	35.6 (33.0; 38.2)	33.1 (29.4; 36.9)	37.8 (34.2; 41.5)
Screen detected diabetes	–	11.4 (9.7; 13.2)	8.0 (6.0; 10.4)	14.4 (11.9; 17.2)
BMI (kg/m^2)	1326	27.1 (4.5)	26.6 (5.1)	27.5 (3.9)
Systolic blood pressure (mmHg)	1324	133 (17)	130 (18)	136 (16)
Diastolic blood pressure (mmHg)	1324	81 (10)	81 (10)	82 (10)
Fasting plasma glucose (mmol/L)	1326	6.0 (0.8)	5.9 (0.7)	6.1 (0.8)
2-h plasma glucose (mmol/L)	1325	6.8 (2.3)	6.6 (2.1)	7.0 (2.4)
Fasting serum insulin (pmol/L)	1325	37 (25; 55)	36 (24; 50)	39 (26; 60)
2-h serum insulin (pmol/L)	1324	184 (110; 313)	185 (117; 299)	181 (101; 325)
$ISI_{0\text{-}120}$	1321	36.9 (25.9; 48.8)	37.2 (27.6; 48.8)	36.4 (24.0; 49.2)
Insulinogenic index	1314	8.3 (5.2; 13.7)	9.0 (5.3; 14.4)	7.9 (5.2; 13.1)
Physical activity measures				
PAEE (kJ/kg/day)	1267	32.7 (15.6)	31.0 (14.8)	34.3 (16.1)
VPA (h/day)	1104	0.00 (0.00; 0.01)	0.00 (0.00; 0.00)	0.00 (0.00; 0.02)
MVPA (h/day)	1104	0.47 (0.19; 0.90)	0.37 (0.16; 0.76)	0.54 (0.22; 1.02)
MVPA ≥0.5 h/day (%)	1104	33.5 (29.8; 37.4)	38.9 (36.3; 41.6)	43.7 (40.0; 47.4)
LPA (h/day)	1104	4.63 (3.39; 5.90)	4.61 (3.30; 5.92)	4.67 (3.41; 5.85)
Sedentary (h/day)	1104	12.2 (2.4)	12.3 (2.4)	12.1 (2.3)
CRF (ml O_2/kg/min)	793	29.9 (5.4)	28.7 (5.1)	30.8 (5.4)
Biochemical measures				
Plasma GLP-1, $tAUC_{0\text{-}30}$ (pmol/L × min)	1310	615 (450; 825)	645 (465; 870)	585 (435; 780)
Plasma GLP-1, $tAUC_{0\text{-}120}$ (pmol/L × min)	1303	2805 (2040; 3795)	3075 (2280; 4200)	2595 (1935; 3480)
Plasma GLP-1, $rAUC_{0\text{-}30}$ (fold increase)	1310	1.7 (1.3; 2.5)	1.9 (1.5; 2.9)	1.6 (1.3; 2.1)
Plasma GLP-1, $rAUC_{0\text{-}120}$ (fold increase)	1303	2.0 (1.5; 3.1)	2.3 (1.7; 3.7)	1.8 (1.4; 2.6)

Data are the means (S.D.), medians (interquartile range) or percentages (95% CI).
CRF, cardiorespiratory fitness; GLP-1, glucagon-like peptide-1; $ISI_{0\text{-}120}$, insulin sensitivity index; LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; NGT, normal glucose tolerance; PAEE, physical activity energy expenditure; VPA, vigorous physical activity.

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increase in MVPA (Table 2). In men, higher PAEE and CRF levels were also associated with lower fasting GLP-1 concentrations (−0.9; −0.1%, P=0.008 and −3.3; −0.5%, P=0.010, respectively). Again, this was not found in women (Table 2).

Glucose-stimulated response of GLP-1

In men, but not in women, the glucose-stimulated GLP-1 responses were 15.8% (0.8; 33.0%, P=0.038) ($rAUC_{0\text{-}30}$) and 20.0% greater (2.6; 40.3%, P=0.022) ($rAUC_{0\text{-}120}$) for every 60-min increase in MVPA, respectively (Table 2). No significant associations between glucose-stimulated GLP-1 responses and PAEE or CRF levels were found in men or women (Table 2).

The results for MVPA and PAEE were replicated in the sensitivity analysis including only the subset of 793 participants with CRF data available (55% men) (Supplementary Table 2).

Discussion

This is to our knowledge the first study in which the association of habitual physical activity with GLP-1 responses during a 2-h OGTT has been investigated in an elderly population at risk of developing T2D. In men, but not in women, we found that the GLP-1 peak response (30 min) to oral glucose was 16% greater and the full response (120 min) was 20% greater for every additional hour spent on moderate-intensity physical activity. These associations were independent of BMI and insulin sensitivity. Furthermore, fasting concentrations of GLP-1 in plasma were lower for every additional hour spent on moderate-intensity physical activity per day. The lower fasting concentrations of GLP-1 were associated with higher cardiorespiratory fitness level (CRF) and higher total activity volume (PAEE). Importantly, almost none of the participants spent time performing vigorous-intensity physical activity, indicating that habitual physical activity at moderate intensity, which in this cohort includes daily chores such as cleaning, gardening, and playing with (grand)children (34), may be sufficient to lower fasting levels and stimulate the glucose-induced GLP-1 response in an elderly population of overweight men.

GLP-1 reduces blood glucose levels after oral glucose intake by stimulating insulin secretion (47), and greater levels of physical activity are associated with substantially lower incidence of T2D (32) and greater insulin sensitivity (34). Fasting and 2-h OGTT levels of glucose are lower with higher maximal oxygen consumption (VO_2 max levels), and glucose-stimulated insulin secretion is inversely associated with VO_2 max, indicating an improved insulin secretion and sensitivity in individuals with higher

Table 2 Associations of GLP-1 levels in plasma and physical activity parameters.

	Model	PAEE		MVPA		CRF[a]	
		Difference	*P*	Difference	*P*	Difference	*P*
Women (*n* = 623)							
Fasting plasma GLP-1 (% change)	1	0.0 (−0.5; 0.5)	0.923	−12.8 (−32.0; 11.7)	0.278	0.5 (−1.4; 2.5)	0.588
	2	0.1 (−0.4; 0.6)	0.654	−10.7 (−30.4; 14.5)	0.372	0.7 (−1.4; 3.0)	0.500
$rAUC_{0\text{-}30}$ (% change)	1	0.2 (−0.2; 0.6)	0.304	−3.5 (−20.2; 16.8)	0.716	0.1 (−1.4; 1.7)	0.862
	2	0.0 (−0.4; 0.4)	0.898	−7.0 (−23.0; 12.4)	0.452	−0.6 (−2.3; 1.1)	0.463
$rAUC_{0\text{-}120}$ (% change)	1	0.2 (−0.2; 0.7)	0.252	−3.1 (−21.5; 19.5)	0.766	0.2 (−1.5; 2.0)	0.784
	2	0.0 (−0.4; 0.4)	0.912	−7.8 (−25.0; 13.3)	0.440	−0.8 (−2.7; 1.1)	0.391
Men (*n* = 703)							
Fasting plasma GLP-1 (% change)	1	−0.6 (−1.0; −0.3)	<0.001	−20.5 (−34.0; −4.4)	0.015	−2.0 (−3.3; −0.7)	0.004
	2	−0.5 (−0.9; −0.1)	0.008	−19.5 (−33.0; −3.3)	0.021	−1.9 (−3.3; −0.5)	0.010
$rAUC_{0\text{-}30}$ (% change)	1	0.2 (−0.1; 0.4)	0.217	16.4 (1.2; 33.8)	0.033	0.0 (−1.0; 1.1)	0.945
	2	0.1 (−0.2; 0.4)	0.640	15.8 (0.8; 33.0)	0.038	−0.3 (−1.4; 0.8)	0.581
$rAUC_{0\text{-}120}$ (% change)	1	0.3 (0.0; 0.6)	0.090	21.0 (3.4; 41.6)	0.018	0.5 (−0.7; 1.7)	0.441
	2	0.1 (−0.2; 0.4)	0.454	20.0 (2.6; 40.3)	0.022	0.0 (−1.2; 1.3)	0.987

Estimated percentage change (95% CI) in fasting and glucose-stimulated response GLP-1 in plasma by a unit increase in PAEE (kJ/kg/day), MVPA (h/day), and CRF (mL O_2/kg/min). Data are percentage change with 95% CI. *P: P* value for test of significance of the association. Model 1: Adjusted for age. Model 2: Further adjusted for BMI and peripheral insulin sensitivity ($ISI_{0\text{-}120}$). MVPA is further adjusted for PAEE so an increase in MVPA is at the expense of a decrease in a less intensive physical activity (MET ≤3.0).

[a]Analyses of cardiorespiratory fitness levels are based on the subset of participants with CRF data available (*n* = 793).

CRF, cardiorespiratory fitness; GLP-1, glucagon-like peptide-1; MVPA, moderate-to-vigorous physical activity; PAEE, physical activity energy expenditure; $rAUC_{0\text{-}30}$, peak glucose-stimulated response 30 min after glucose ingestion; $rAUC_{0\text{-}120}$, full glucose-stimulated response 120 min after glucose ingestion.

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https://doi.org/10.1530/EC-19-0408

cardiorespiratory fitness levels (35, 48, 49). Moreover, in another study on the present cohort, higher levels of PAEE were positively associated with insulin sensitivity and negatively with circulating insulin 2 h after glucose load (34). Therefore, beta-cells of individuals with higher physical activity levels seem to be 'sensitized' to secrete the minimum amount of insulin required for accurate glycaemic control (35, 48, 49) and therefore fasting levels are lower with higher activity levels.

Interestingly, in the present study, we observe that, independent of insulin sensitivity, greater moderate-intensity physical activity is associated with lower fasting and greater glucose-stimulated GLP-1 response in men. Our findings suggest that a 'sensitizing effect' of physical activity exists for the GLP-1 secreting cells, which keeps fasting GLP-1 levels at a minimum whilst having an enhanced ability to acutely respond to nutrient intake with greater postprandial GLP-1 secretion. The effect of increased GLP-1 responses might be to sensitize the beta-cell to glucose and thus the beta-cell produces the same amount of insulin but at lower plasma glucose levels. The exact underlying mechanisms for physical activity to affect GLP-1 secretion from intestinal L-cells remain unknown, but differences in gastrointestinal motility (10, 12) and gastric emptying (50, 51) could be involved.

No associations were found in the women, who were less physically active than men

We did not observe similar associations in women, which may be because the women in this study may not be sufficiently physically active in their everyday life. Only 39% of the women included in the analyses met the Danish recommendations for moderate-intensity physical activity (i.e. ≥30 min/day). The average differences of daily physical activity between men and women corresponded to approximately 10 min/day (70 min/week). The women also had lower cardiorespiratory fitness levels. Sex specificity in terms of GLP-1 secretion has previously been established (6, 52) and a genetic component for cardiorespiratory fitness may partly explain some difference in fitness levels between sexes (53). Moreover, increased adiposity in women may also have an impact on fitness levels when presented relative to body weight, that is, for a similar body weight, women may have lower metabolically active lean tissue. In relation to this, the inhibiting effects of free fatty acids on GLP-1 secretion (29, 30) could potentially explain why GLP-1 responses are lower in women whose levels of free fatty acids in blood might be increased compared to men.

Comparison with other studies of GLP-1 and physical activity

A few longitudinal studies have investigated the medium- and long-term effects (≥3 months) of regular exercise interventions on GLP-1 secretion in overweight individuals (18, 25, 26). In a recent randomized controlled trial, Quist and colleagues reported higher fasting and postprandial GLP-1 concentrations after 6 months of vigorous exercise 5 days/week (25), and in a comparable study from 2007, Martins *et al.* found a tendency towards an increase in the delayed (90–180 min) postprandial GLP-1 response in overweight individuals after 12 weeks of regular exercise at vigorous intensity (18). These increased postprandial responses are in line with our findings, although we found that moderate intensity is enough for physical activity to be associated with greater GLP-1 response to glucose. However, Quist *et al.* found that neither active commuting nor exercise at moderate intensity affected GLP-1 secretion (25). Also, the findings of increased fasting levels of GLP-1 after 6 months of regular vigorous exercise are in contrast to our findings, where we show fasting levels to be lower with more time spent being physically active. Explanations for contradictory fasting levels between studies may be the difference in age of the participants (i.e. 66 years in this present study and 34 years in the study of Quist) or that the prescribed medium-term vigorous exercise intervention was insufficient to 'sensitize' intestinal secretion.

Strengths, limitations, and unanswered questions of the present study

Besides the large number of included participants (n=1326), the study has several strengths using robust methods for quantification and analysis of physical activity parameters and GLP-1 levels: in regards to physical activity parameters, a strength is that physical activity behaviour is objectively assessed by 7 days of combined heart rate monitoring and accelerometry that have shown to give reliable estimates of physical activity (54) superior to subjective methods, or heart rate monitors or accelerometers alone (55). Also, the estimation of cardiorespiratory fitness levels based on step tests with individual heart rate monitoring is a strength (39). An additional strength is the adjustment of total activity volume – expressed as physical activity energy expenditure – in the regression analyses of moderate-intensity physical activity and GLP-1 to ensure that an increase in moderate-intensity physical activity is at the expense of a reduction

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in a less intensive physical activity (≤3 METs) and not due to an increase in total activity volume.

As briefly mentioned, the quantification method of GLP-1 is essential for the comparability between clinical studies investigating GLP-1 secretion (28). In the present study, we measured total GLP-1, whereas several studies report concentrations of only active or intact GLP-1 (primarily GLP-1(7-36)NH2) that is secreted into the capillaries draining the small intestine. However, active GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) to form the GLP-1 metabolite (9-36NH2) and therefore only approximately 8% of the GLP-1 that was secreted may reach target organs like the pancreas (56). However, newly secreted GLP-1 appears to interact with sensory nerves in the lamina propria (on its way from the L-cell to the capillaries, where it starts to be degraded). It is therefore important to measure the total amount of GLP-1 secreted, and not only the small fraction that survives in the intact form in peripheral plasma. Therefore, measuring the total GLP-1 (i.e. both 7-36NH2 and 9-36NH2) better reflects not only the secretion from the L-cell but also the sum of its neuronal and endocrine actions (28).

A limitation of the present study, besides the cross-sectional design, is that the men and women were not matched with regards to the amount of time spent on moderate-intensity physical activity (weekly difference of 70 min per week between sexes). Future studies including men and women equally matched in terms of time spent on moderate-intensity physical activity may be needed for further clarification of a sex-specific difference in the association between GLP-1 response and physical activity. Also, conducting a longitudinal study of the effects of habitual physical activity on GLP-1 secretion may elucidate potential causality in the association.

Clinical implications of the study

Findings of the present study indicate that habitual physical activity at moderate intensity have positive associations with glucose-stimulated GLP-1 secretion, independent of insulin sensitivity, in elderly men at risk of type 2 diabetes. Greater postprandial GLP-1 responses may not only be beneficial for glucose control (through stimulation of insulin secretion) but also for appetite inhibition and satiety sensation (8, 57) which is beneficial in terms of prevention and treatment of overweight. Examples of activities requiring moderate intensity effort include brisk walking, gardening, walking domestic animals, and active involvement in games with grandchildren (58) all of which could be incorporated in the everyday lives of most people. A cohort study in 334,000 Europeans from 2015 showed that all-cause mortality rates could be reduced by 7% if all inactive individuals increased their activity levels to the equivalent of at least 20-min brisk walking per day (59). For comparison, avoiding obesity (BMI>30) only reduces the number of deaths by 3% (59). Thus, focus on public health advises that even moderate-intensity activities are associated with improved metabolic and overall health is warranted.

Conclusion

In 703 men, we observed that more time spent being physically active was associated with lower fasting and greater glucose-induced GLP-1 responses, independent of insulin sensitivity. This indicates a beneficial effect of increasing time spent on even moderate-intensity habitual physical activity on GLP-1 secretion, which could contribute to improved glucose regulation and reduce the risk of type 2 diabetes. This was not observed in women, who were less physically active. Future mechanistic studies are needed to explore the potential molecular mechanism(s) underlying how habitual physical activity may affect fasting and glucose-induced GLP-1 secretion in humans.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/EC-19-0408.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

C J was supported by a research grant from the Danish Diabetes Academy (DDA) funded by the Novo Nordisk Foundation (NNF) during this work. Furthermore, this work was supported by the Tripartite Immunometabolism Consortium (TrIC) Novo Nordisk Foundation; Grant number NNF15CC0018486. K F was supported by the Novo Nordisk Foundation during this work. S B was supported by the UK Medical Research Council (MC_UU_12015/3) and the NIHR Biomedical Research Centre Cambridge (IS-BRC-1215-20014). For the remaining authors none were declared.

Author contribution statement

Conceptualization S S T, K F. Statistical analyses were performed by D V. S B modelled PAEE from raw data. S S T, K F, D V and C J interpreted data. C J drafted the manuscript with help from S S T. D R W and T L designed the

ADDITION-PRO study with contribution from T H, S B and O P. GLP-1 data were analysed at the laboratory of J J H. K F and S S T are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to completion of the manuscript including critical revision and all authors approve the manuscript. K Færch and S S Torekov shared co-last authors.

Acknowledgments

The authors acknowledge the ADDITION-PRO study centers, the staff, and the participants for their important contribution to the study. The authors also thank the laboratory technicians Lene Albæk and Sofie Olsen, Holst laboratory, University of Copenhagen. They thank Nicolai J Wewer Albrechtsen (Department of Clinical Biochemistry, Rigshospitalet, Copenhagen) for help and discussions.

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Received in final form 14 November 2019
Accepted 21 November 2019